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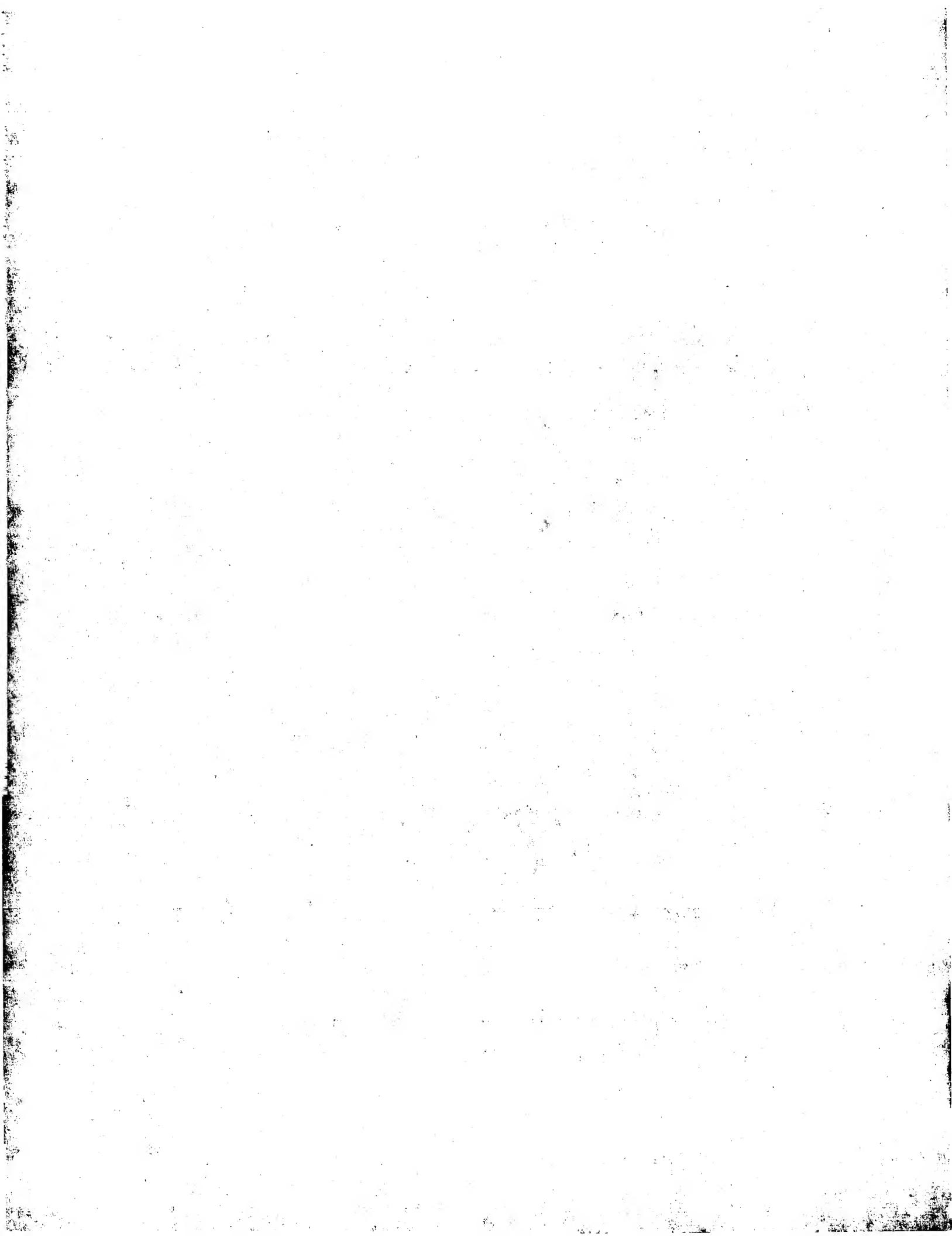
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(54) Title: POLYENE MACROLIDE PRE-LIPOSOMAL POWDERS

(57) Abstract

The present invention involves a process for producing fine powder suitable for the preparation of antifungal polyene macrolide-containing liposomes upon suspension in an aqueous solution. This process comprises the following steps. Quantities of polyene macrolide and phospholipids are dissolved respectively in a first solvent and a second solvent to form a first solution and a second solution. The first solution and the second solution are mixed in a desired ratio to form a mixture. The first solvent and the second solvent are then removed from the mixture, for example by evaporation, to form a residue. The residue is then dissolved in a third solvent comprising tertiary butanol and methylene chloride to form a third solution. The third solvent is then removed from the third solution to form a remnant. The remnant is then dissolved in a solvent consisting essentially of tertiary butanol to form a fourth solution. The fourth solution is then filtered through a filter having orifices of between about 0.05 and 0.5 micrometers in diameter to produce a filtrate. The filtrate is lyophilized to remove the tertiary butanol and a fine powder remains. This fine powder may be used to form polyene macrolide-containing liposomes by simple incubation or suspension in an aqueous solution.

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Polyene macrolide pre-liposomal powders.

BACKGROUND OF THE INVENTION

The present invention relates to a composition of matter usable to form liposomes comprising antifungal 5 polyene macrolides and the production thereof.

Clinical observations and animal experimental studies have added to the understanding of host-fungal interactions. It is becoming recognized that host defense 10 against fungal disease is multifactorial and may vary, depending on the etiologic agent. The mechanisms of resistance are not well defined in most instances, but various innate barriers and cell-mediated immune responses seem to be of primary importance. Clearly, debilitation 15 of innate defenses and of cell-mediated immune responses can increase an individual's susceptibility to severe fungal disease from opportunistic agents such as Cryptococcus neoformans and species of Candida and Aspergillus, as well as from fungal pathogens such as Histo- 20 plasma capsulatum and Coccidioides immitis. The difficulty in gaining a complete understanding of the critical host defenses has been further complicated by many studies that show fungi may affect various host immune functions adversely. Although it is too early to evaluate the 25 clinical importance of many of these experimental findings, investigators have demonstrated that fungi impair neutrophil function, induce IgE responses, and cause suppression of cell-mediated immune responses.

30 Host changes likely to be associated with increased susceptibility may be accidentally induced, as in traumatic injuries (such as burns or puncture wounds); self-induced, as in chronic alcoholism; naturally occurring, as in diabetes mellitus, various congenital immune deficien-

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cies, collagen diseases, lymphoreticular neoplastic disease, and other types of tumors; or iatrogenically induced by instrumentation (such as catheterization), surgical procedures (such as open heart surgery), or by 5 use of cytotoxic drugs (as in an attempt to prevent graft rejection and to treat neoplastic disease), corticosteroid therapy, and long-term use of broad-spectrum antibiotics.

Chemical factors that aid resistance to fungal 10 diseases are poorly defined. Knowledge of these substances is based primarily on circumstantial evidence at the clinical level and in vitro observations at the experimental level. Hormonally associated increases in lipid and fatty acid content on the skin occurring at 15 puberty have been correlated with increased resistance to tinea capitis caused by the dermatophyte Microsporum audouinii, although pubescent changes are not the sole factors in resistance. Substances in serum, cerebrospinal fluid, and saliva may limit growth of Cryptococcus neoformans, and basic peptides in body fluids have been shown to 20 inhibit Candida albicans.

Results of clinical and experimental studies indicate that C. albicans, C. neoformans, Aspergillus fumigatus, 25 and C. immitis activate the alternative pathway of the complement cascade. Because of the polysaccharide nature of fungal cell walls, it is expected that all medically important fungi activate complement. Such activation may be important in defense against some mycoses; a positive 30 correlation has been demonstrated between animals deficient in late-acting complement components (C3-C9) and increased susceptibility to fungi such as C. neoformans and C. albicans. Assuming that phagocytic cells are important in resistance to fungi, complement activation

may play a role by provoking an acute inflammatory response on generation of complement fragments C3a and C5a, and by coating the fungal elements with opsonic fragments C3b and C3d for ingestion by phagocytic cells.

5

The systemic mycoses of humans and other animals are caused by some fungi that are pathogenic and cause disease in the healthy host, and by other fungi (opportunistic pathogens) that are usually innocuous but cause disease in 10 patients whose immune defenses are impaired. Some of these fungi may be saprophytes in nature (soil, bird droppings), whereas others are a part of the normal human flora (commensals). In no case are humans the solitary or necessary host.

15

An example of a soil saprophyte is Histoplasma capsulatum, which commonly causes infection in endemic areas; 80%-90% of adults react positively to histoplasmin in delayed cutaneous hypersensitivity tests. An example 20 of an opportunistic pathogen is Candida albicans, normally present in the oral cavity, gastrointestinal tract, and probably the skin. In the patient with acute leukemia, however, C. albicans is commonly present in blood, causing a fulminant, usually fatal, septicemia. Other opportunistic infections are seen in patients with diabetic acidosis (mucormycosis) and Hodgkin's disease (for example, cryptococcosis and histoplasmosis). The pathogenesis of these mechanisms is obscure, but cell-mediated immunity seems to be essential for a good prognosis.

25

30 Neither active vaccines nor passive immune serum immunization has been sufficiently successful to result in commercially available preparations.

Treatment of active disease may be symptomatic (for example, pain relief), sometimes surgical (resection of irremedially damaged tissue and correction of hydrocephalus), and, most successfully, chemotherapeutic (Table 1). Among the chemotherapeutic agents commonly used are hydroxystilbamidine isethionate, amphotericin B, 5-fluorocytosine (Flucytosine), miconazole, and ketoconazole. Response to these drugs varies according to the fungus, type of disease, and course of illness. For example, response is good in most *B. dermatitidis* infections, but is poor in most diseases caused by *A. fumigatus*. Response is better for skin lesions caused by *B. dermatitidis* than for meningitis due to *C. immitis*; response is better in chronic cryptococcosis than in fulminant candidiasis. Table 1 shows a listing of some systemic mycoses and generally accepted chemotherapeutic agents.

TABLE 1

5

CHEMOTHERAPEUTIC AGENTS FOR SYSTEMIC MYCOSES

	Disease	First Choice	Second Choice
10	Aspergillosis	Amphotericin B	Ketoconazole
	Blastomycosis	Amphotericin B	Hydroxystilbamidine isethionate
15	Candidiasis	Amphotericin B	Flucytosine or ketoconazole
	Coccidioidomycosis	Amphotericin B	Ketoconazole
	Cryptococcosis	Amphotericin B Flucytosine	Either drug alone*
	Histoplasmosis	Amphotericin B	Ketoconazole*
20	Mucormycosis	Amphotericin B	Miconazole*
	Paracoccidioidomycosis	Amphotericin B	Sulfonamides, Ketoconazole*

25 *Depending on minimal inhibitory concentration necessary for the fungus.

Infection is the cause of death in 51% of patients with lymphoma and 75% of patients with leukemia. Although bacteria are the causative organisms of many such infections, fungi account for 13% of the fatal infections in patients with lymphoma and for more than 20% of patients with leukemia. The fungus Candida albicans causes more than 80% of these infections, and Aspergillus spp. is also a frequent cause of such infections. In addition, fungal infection is a major cause of morbidity and mortality in patients with congenital and acquired deficiencies of the immune system. Much concerted effort has been expended in search of agents useful in treating fungal infections of humans. As a result, many compounds have been isolated and shown to have antifungal activity, but problems associated with solubility, stability, absorption, and toxicity have limited the therapeutic value of most of them in human infections. The most useful antifungal antibiotics fall into one of two categories: those that affect fungal cell membranes and those that are taken up by the cell and interrupt vital cellular processes such as RNA, DNA, or protein synthesis. Table 2 lists some useful antifungal agents and their mechanisms of action.

TABLE 2

SOME USEFUL ANTIFUNGAL AGENTS, THEIR CHEMICAL CLASSIFICATION, AND THEIR MECHANISMS OF ACTION

5

	Class	Compounds	Mechanism
10	Polyene	Amphotericin B Nystatin	Interacts with sterols (ergosterol) in fungal cell membrane, rendering cells selectively permeable to the outflow of vital constituents, e.g. potassium
15			
20	Imidazole	Miconazole Clotrimazole Ketoconazole	Inhibits demethylation of lanosterol thus preventing formation of ergosterol, a vital component of fungal cell membrane; also has a direct cidal effect on fungal cells
25			
30	Pyrimidine	5-Fluorocytosine	Is taken up and deaminated by susceptible cell to form 5-fluorouracil, which in turn inhibits RNA synthesis; also thought to inhibit thymidylate synthetase and DNA synthesis
35			
40	Grisan	Griseofulvin	Binds to tubulin and inhibits microtubule assembly
45	3-Arylpyrrole	Pyrrolnitrin	Appears to inhibit terminal electron transport between succinate or NADH and coenzyme Q
50	Glutaramide	Cycloheximide	Inhibits protein synthesis at 80S ribosomal level preventing transfer of aminoacyl tRNA to the ribosome

The polyene macrolide antibiotics are secondary metabolites produced by various species of Streptomyces. Several common features of these compounds are useful in 5 classifying the more than 80 different polyenes that have been isolated. All are characterized by a macrolide ring, composed of 26-38 carbon atoms and containing a series of unsaturated carbon atoms and hydroxyl groups. These features of the molecule contribute to the polyenes' 10 amphipathic properties (those relating to molecules containing groups with different properties, for example, hydrophilic and hydrophobic). The ring structure is closed by the formation of an internal ester or lactone bond (Figure 1). The number of conjugated double bonds 15 vary with each polyene, and the compounds are generally classified according to the degree of unsaturation.

Toxic effects of polyene macrolides appear to be dependent on binding to cell membrane sterols. Thus, they 20 bind to membranes of fungus cells as well as to those of other eukaryotic cells (human, plant, and protozoa), but not to bacterial cell membranes, which do not contain membrane sterols. The interaction of polyene macrolides with mammalian and fungal membrane sterols results in 25 transmembrane channels that allow the leakage of intracellular components leading to cell deaths.

The usefulness of an antibiotic is usually measured by the differential sensitivity of the pathogen and host. 30 Two polyene macrolides agents, nystatin and amphotericin B, are relatively specific for fungi and have thusfar proven to have therapeutic usefulness in humans. The relative specificity of these two polyene macrolides may be based on their greater avidity for ergosterol, the

principal sterol of fungal membranes, compared to cholesterol, the principal sterol of human cell membranes.

Amphotericin B is a heptaene macrolid with seven 5 resonating carbon bonds. The compound was first isolated from broth filtrates of S. nodosum in 1956. Like other polyene macrolide antibiotics, amphotericin B is insoluble in water. The problem of its solubility has been circumvented by combining the antibiotic with sodium deoxycholate and sodium phosphate and hydrating the mixture with 10 sterile water or saline. Amphotericin B is the polyene antibiotic thusfar most sufficiently nontoxic to humans that it has been used parenterally at effective doses against various fungi.

15

Nystatin, first isolated from S. noursei, is structurally related to amphotericin B, but is not classified as a heptaene because the conjugated portion of the ring is interrupted and thus forms a tetraene and a diene. 20 Tolerated well both orally and topically, the drug is not available for intravenous use because of its presumed high toxicity and aqueous insolubility. Nystatin is available as oral tablets (500,000 units) or as an ointment for topical use (100,000 units/g). It is used in the management of 25 cutaneous and mucocutaneous candidiasis.

It has recently been shown that the encapsulation of certain drugs in liposomes before administration to the patient can markedly alter the pharmacokinetics, tissue 30 distribution, metabolism and therapeutic efficacy of these compounds. Liposomes may be defined as lipid vesicles which are formed spontaneously on addition of an aqueous solution to a dry lipid film. Further, the distribution and pharmacokinetics of these drugs can be modified by

altering the lipid composition, size, charge and membrane fluidity of the liposome in which they are encapsulated.

Recently, liposomes have been used as carriers of

5 amphotericin B for treatment of murine leishmaniasis (New, R.R.C., et al., "Antileishmanial Activity of Amphotericin and Other Antifungal Agents Entrapped in Liposomes." J. Antimicrob. Chemother., Vol. 8 (1981), pp. 371-381), histoplasmosis (Taylor, R.L., et al., "Amphotericin B in

10 Liposomes: A Novel Therapy for histoplasmosis." Am. Rev. Respir. Dis., Vol. 125 (1982), pp. 610-611), cryptococcosis (Graybill, J.R., et al., "Treatment of Murine Cryptococcosis with Liposome-Associated Amphotericin B." J. Infect. Dis., Vol. 145 (1982), pp. 748-752). and candidiasis

15 (Tremblay, C., et al., "Comparative Efficacy of Amphotericin B (AMB) and Liposomal AMB (lip-AMB) in Systemic Candidiasis in Mice." Abstr. 1983 ICAAC, No. 755 (1983), p. 222). Liposome-encapsulated Amphotericin B has also been used for treatment of coccidioidomycosis in the

20 Japanese macaque (Graybill, J.R., et al., "Treatment of Coccidioidomycosis (coccidioidomycosis) in Primates Using Liposome Associated Amphotericin B (Lipo-AMB)." Abstr. 1982 ICCAC, No. 492 (1982), p. 152).

25 The treatment of fungal infections remains a major problem in spite of the availability of effective anti-fungal drugs such as the polyenes. Most of the available polyene antibiotics have toxic side effects that limit their clinical application. Nystatin, a tetraene-diene

30 polyene macrolide antibiotic, has high hydrophobicity, which has precluded its effective systemic administration. It has been used as suspensions prepared in various ways and administered to the patients orally. However, these studies have generally failed to document a beneficial

effect of nystatin administration against systemic fungal infections.

The present inventors have recently demonstrated that 5 liposome-encapsulated amphotericin B may be used to treat experimental murine candidiasis (Lopez-Berestein *et al.*, J. Infect. Dis., Vol. 150, pp 278-283 (1984) and in the treatment of fungal infections in patients with leukemia and lymphoma (Lopez-Berestein *et al.*, J. Infect. Dis., 10 Vol. 151, pp 704-71- (1985)).

SUMMARY OF THE INVENTION

15 The present invention involves a process for producing fine powder suitable for the preparation of antifungal polyene macrolide-containing liposomes upon suspension in an aqueous solution. This process comprises the following steps. Quantities of polyene macrolide and 20 phospholipids are dissolved respectively in a first solvent and a second solvent to form a first solution and a second solution. The first solution and the second solution are mixed in a desired ratio to form a mixture. The first solvent and the second solvent are then removed 25 from the mixture, for example by evaporation, to form a residue. The residue is then dissolved in a third solvent comprising tertiary butanol and methylene chloride to form a third solution. The third solvent is then extracted by evaporation from the third solution to form a remnant. 30 The remnant is then dissolved in a solvent consisting essentially of tertiary butanol to form a fourth solution. The fourth solution is then filtered through a filter having orifices of between about 0.05 and 0.5 micrometers in diameter to produce a filtrate. The filtrate is

lyophilized to remove the tertiary butanol and a fine powder remains. This fine powder may be used to form polyene macrolide-containing liposomes by simple incubation or suspension in an aqueous solution.

5

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

A stable powder suitable for the direct preparation 10 of liposome-incorporated antifungal polyene macrolides may be made by a process of the present invention. While the conditions described herein are specifically applicable to nystatin and amphotericin B, other polyene macrolide antifungals may be likewise used, but with minor modifications 15 of procedure apparent to those skilled in the art upon a minimal amount of experimentation.

The process for pre-liposomal polyene macrolide powder formation of the present invention involves 20 dissolution of an antifungal polyene macrolide such as nystatin or amphotericin B in a first organic solvent such as methanol to form a first solution. Phospholipids are dissolved in a second organic solvent such as, for example, chloroform, to form a second solution. The first 25 solution and the second solution are mixed to form a first mixture having a ratio of antifungal polyene macrolide to phospholipid between about 1:5 and about 1:50, preferably of about 1:10. The organic solvents are removed from the mixture, for example, by solvent evaporation under reduced 30 pressure and at a temperature between about 35°C and about 45°C, until a residue such as a dry film is formed. The residue is then dissolved in a quantity of a third organic solvent such as a mixture of tertiary butanol and methylene chloride in a ratio between about 2:1 (preferred for

nystatin) and about 1:40 (preferred for amphotericin B) and the solvent evaporated to leave a remnant. The remnant is dissolved in a solvent consisting essentially of tertiary butanol to form a fourth solution which is 5 warmed, if necessary for clarification, and passed through a filter having orifices of between about 0.05 and 0.5 micrometers (um) in diameter. If warming is desired to clarify the fourth solution, particularly with amphotericin B, the warming is preferably to a temperature 10 between about 50°C and about 70°C. The filtrate is subjected to freezing, for example, with dry ice-acetone. The frozen material is then lyophilized until essentially all solvent is removed. After lyophilization, a fine 15 pre-liposomal polyene macrolide powder is produced. This powder is readily and stably stored under commonly available dry and cool storage conditions.

The above-described pre-liposomal polyene macrolide powder may be easily used to reconstitute a liposome 20 suspension according to the following general procedure. The powder is added to an aqueous solution such as pyrogen-free saline, and allowed to incubate at 25°C to 45°C for 1-10 minutes for a liposome suspension to form. Polyene macrolide content may be measured by dissolution 25 of the liposomes in methanol and monitoring of optical density at a wavelength characteristic for polyene macrolide absorption.

Representative, suitable phospholipids in the present 30 invention are phosphatidylcholine, both naturally occurring and synthetically prepared, phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, sphingolipids, phosphatidylglycerol, sphingomyelin, cardiolipin, glycolipids, gangliosides, cerebrosides and the like used

either singularly or intermixed such as in soybean phospholipids.

More particularly useful phospholipids include egg 5 phosphatidylcholine, dilaurylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, 1-myristoyl-2-palmitoylphosphatidylcholine, 1-palmitoyl-2-myristoylphosphatidylcholine, 1-stearoyl-2-palmitoyl phosphatidyl-10 choline, dioleoylphosphatidylcholine, dilauryloylphosphatidylglycerol, dimyristoylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, distearoylphosphatidylglycerol, dioleoylphosphatidylglycerol, dimyristoyl phosphatidic acid, dipalmitoyl phosphatidic, dimyristoyl 15 phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, dimyristoyl phosphatidylserine, dipalmitoyl phosphatidylserine, brain phosphatidylserine, brain sphingomyelin, dipalmitoyl sphingomyelin, and distearoyl sphingomyelin.

20

The lipid composition of both the initial powdered composition of matter and the resultant liposomes, formed in accordance with the present method, is normally the same. Where the resultant liposomes are intended for 25 in vivo applications (such as drug delivery), then it is normally desirable that the lipid composition have a transition temperature below body temperature. Liposomes composed of phospholipids which have transition temperatures below the characteristic gel-liquid 30 crystalline phase transition temperature of biological membranes, i.e. about 37°C, are considered fluid and those which have transition temperature above 37°C are considered solid. Another consideration in selecting the composition of lipid or lipids for liposome applications

is that alkyl-ether linked lipids (rather than ester linked) are more stable to hydrolysis, and hence alkyl-ether linked lipids for the resultant liposomes may be particularly desirable for therapeutic application.

5

In addition, other lipid-like substances such as steroids, cholesterol, aliphatic amines or acids such as long chain aliphatic amines or carboxylic acids, long chain sulfates and phosphates, dicetyl phosphate, 10 butylated hydroxytoluene, tocopherol, and isoprenoid compounds may be intermixed with the phospholipid components to confer certain desired and known properties on the initial liposomes and hence the resultant liposomes. Further, synthetic phospholipids containing 15 either altered aliphatic portions, such as hydroxyl groups, branched carbon chains, cycloderivatives, aromatic derivatives, ethers, amides, polyunsaturated derivatives, halogenated derivatives, or altered hydrophilic portions containing carbohydrate, glycol, phosphate, phosphonate, 20 quaternary amine, sulfate, sulfonate, carboxy, amine, sulfhydryl, imidazole groups and combinations of such groups, can be either substituted or intermixed with the phospholipids.

25

The antifungal polyene macrolides of the present invention include nystatin, amphotericin B, partricin and derivatives thereof such as methyl esters.

30

These examples are presented to illustrate preferred embodiments and utilities of the present invention and are not meant to limit the present invention unless otherwise stated in the claims appended hereto.

EXAMPLE 1

Preparation and Use of a Pre-Liposomal
Nystatin Powder (L-Nys)

5

A solution of 25 mg nystatin in 25 ml methanol was mixed with a solution of 175 dimyristoylphosphatidyl-choline (DMPC) and 75 mg dimyristoylphosphatidylglycerol (DMPG) in 10 ml chloroform. The DMPC:DMPG ration was 7:3 and the nystatin:DMPC+DMPG ration was 1:10. The organic solvents were then evaporated at 40°C under partial vacuum in a rotary evaporator until a dried lipid film was formed. Thirty ml of 2:1 mixture of tertiary butanol and methylene chloride were added to dissolve the dried lipid film. The organic were then evaporated from the solution at 40°C and under partial vacuum to form a lipid residue. The lipid residue was dissolved in tertiary butanol and the solution passed through a 0.2 um filter. The nystatin concentration was measured from an aliquot of the filtrate. The filtrate was frozen by immersion of a container in dry ice-acetone. The frozen material was subjected to overnight lyophilization and a fine pre-liposomal nystatin powder produced.

25 A 100 mg sample of the fine powder (containing about 10 mg nystatin) was suspended with 10 ml of pyrogen-free saline. When the powder suspension was warmed at 40°C for 2-5 minutes, liposomes were formed therein. As determined by microscopic examination, the suspended materials were 30 100% liposomes were formed therein. As determined by microscopic examination, the suspended materials were 100% liposomes and no crystals were found. The suspension was centrifuged at 20,000 rpm (40,700 x g) for one hour and the resultant pellet removed and resuspended in saline.

The nystatin remaining in the resuspended pellet was determined to be 70-80 percent of the original amount added, by dissolution in methanol and measurement of optical density at 306 nm. The encapsulation efficiency 5 of the liposomes, as measured after the filtration step, was observed to be > 99%. (No detectable free drug was left after formation of liposomes from the powder). The resuspended pellet was a liposome preparation substantially free of soluble lipids or other materials 10 and was suitable for clinical administration.

EXAMPLE 2

15

Preparation and Use of a Pre-Liposomal
Amphotericin B Powder

Amphotericin B in methanol and phospholipids (DMPC:DMPG, 7:3) in chloroform were mixed together in a 20 ratio of 1:10. The organic solvents were then evaporated at 40°C using a rotary evaporator under vacuum.

Tertiary butanol and methylene chloride in a 1:30-40 ratio were added to solubilize the dried lipid film. The 25 organic solvents were then evaporated.

The residue in the flask was then dissolved in tertiary butanol, warmed to temperatures above 52°C, and filtered through a 0.2 um filter. An aliquot from this 30 filtrate was taken to determine the amphotericin B concentration.

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The above mixture was then frozen (using dry ice with acetone) and lyophilized overnight. A fine powder was obtained.

5 The powder obtained as described above was suspended in pyrogen-free saline. The liposomes did not form until the suspension was warmed in a water bath at about 40°C for about 2-5 minutes. The suspension then formed 100% liposomes (no crystals), as they appeared under a microscope. The suspension was centrifuged at 20,000 rpm for one hour and the pellet removed and resuspended in saline. An aliquot was taken from this final suspension and the amount of amphotericin B incorporated into liposomes quantitated by dissolving in methanol and measuring O.D. 15 at 405 nm. The encapsulation efficiency of drug from the powder to liposomes was 99-100%.

* * * * *

20

Changes may be made in the elements and methods described herein or in the steps or the sequence of steps of the method described herein without departing from the 25 concept and scope of the invention as defined in the following claims.

WHAT IS CLAIMED IS:

1. A process for producing a powder which forms
5 liposomes comprising an antifungal polyene macrolide upon
suspension in an aqueous solution, said process comprising
the steps of:
 - (a) dissolving antifungal polyene macrolide and
10 phospholipids in a quantity of first organic
solvent and a quantity of second organic solvent
respectively, to form a first solution and a
second solution;
 - (b) mixing the first solution and the second solution
15 to form a mixture;
 - (c) removing the first organic solvent and the second
organic solvent from the mixture to form a
20 residue;
 - (d) dissolving the residue in a quantity of a third
organic solvent to form a third solution;
 - (e) extracting the third organic solvent from the third
solution to leave a remnant;
25
 - (f) forming a fourth solution by dissolving the remnant
in a solvent consisting essentially of tertiary
butanol;
30
 - (g) passing the fourth solution through a filter having
orifices with diameters of between about 0.1 nm
and about 0.5 nm to produce a filtrate; and

(h) lyophilizing the filtrate to remove the solvent consisting essentially of tertiary butanol.

5 2. A composition of matter produced essentially by the process of claim 1.

10 3. A composition of matter produced by a process comprising the steps of:

15 (a) dissolving antifungal polyene macrolide and phospholipids in a quantity of first organic solvent and a quantity of second organic solvent to form respectively a first solution and a sec nd solution;

20 (b) mixing the first solution and the second solution to form a first mixture;

(c) removing the first organic solvent and the second organic solvent to form a residue;

25 (d) dissolving the residue in a quantity of a third organic solvent to form a third solution;

(e) extracting the third organic solvent from the third solution to leave a remnant;

30 (f) forming a fourth solution by dissolving the remnant in a solvent consisting essentially of tertiary butanol;

(g) passing the fourth solution through a filter having orifices with diameters of between about 0.1 nm and about 0.5 nm to produce a filtrate; and

5 (h) lyophilizing the filtrate to remove the solvent consisting essentially of tertiary butanol.

4. The process of claim 1 or composition of matter of
10 claim 3 wherein the antifungal polyene macrolide is
nystatin, amphotericin B, partricin or a derivative
thereof.

15 5. The process of claim 1 or composition of matter of
claim 3 wherein the antifungal polyene macrolide is
nystatin or amphotericin B.

20 6. The process of claim 1 or composition of matter of
claim 3 wherein the antifungal polyene macrolide is
amphotericin B.

25 7. The process of claim 1 or composition of matter of
claim 3 wherein the antifungal polyene macrolide is
nystatin.

30 8. The process of claim 1 or the composition of matter
of claim 3 wherein the phospholipids are one or more of
phosphatidylcholine, phosphatidylserine, phosphatidyl-
glycerol, sphingomyelin and phosphatidic acid.

9. The process of claim 1 or the composition of matter of claim 3 wherein the phospholipids comprise DMPC and DMPG.

5

10. The process of claim 1 or the composition of matter of claim 3 wherein the phospholipids consist essentially of DMPC and DMPG in 7:3 ratio.

10

11. The process of claim 1 or the composition of matter of claim 3 wherein the first solvent is methanol.

15

12. The process of claim 1 or the composition of matter of claim 3 wherein the second solvent is chloroform.

15

13. The process of claim 1 or the composition of matter 20 of claim 3 wherein step (b) is defined further as:

25

mixing the first solution and the second solution to form a first mixture having a ratio of anti-fungal polyene macrolide to phospholipid between about 1:5 and about 1:50.

30

14. The process of claim 1 or the composition of matter of claim 3 wherein step (b) is defined further as:

mixing the first solution and the second solution to form a first mixture having a ratio of anti-fungal polyene macrolide to phospholipid of about 1:10.

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15. The process of claim 1 or the composition of matter of claim 3 wherein step (c) is defined further as:

5 removing the first solvent and the second solvent from the first mixture by subjecting the first mixture to solvent evaporation under reduced pressure and at a temperature between about 35°C and about 45°C.

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16. The process of claim 1 or the composition of matter of claim 3 wherein, prior to the passing step, the fourth solution is clarified by warming to between about 50°C and about 70°C.

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17. The process of claim 1 or the composition of matter of claim 3 wherein the third organic solvent comprises tertiary butanol and methylene chloride.

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18. The process of claim 1 or the composition of matter of claim 3 wherein the third organic solvent comprises tertiary butanol and methylene chloride in a ratio between 25 about 2:1 and about 1:40.

30 19. The process of claim 1 or the composition of matter of claim 3 defined further wherein the filter has orifices of about 0.2 nm.

20. The process of claim 1 or the composition of matter of claim 3 wherein the antifungal polyene macrolide and

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phospholipids are in a ratio of between about 1:5 and about 1:20.

5 21. The process of claim 1 or the composition of matter of claim 3 wherein the antifungal polyene macrolide and phospholipids are in a ratio of about 1 to 10.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03652

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC ⁴ A 61 K 9/50; A 61 K 31/71

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
IPC ⁴	A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US, A, 4663167 (LOPEZ-BERESTEIN et al.) 5 May 1987 see column 5, lines 1-25 --	
A	FR, A, 2390159 (ICI) 8 December 1978 see page 6, example 1; claims --	
A	EP, A, 0087993 (PARFUMS CHRISTIAN DIOR) 7 September 1983 see pages 25,26, example 12; claims -----	

- Special categories of cited documents: ¹⁶
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

17th February 1989

Date of Mailing of this International Search Report

16 MAR 1989

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

P.C.G. VAN DER PUTTEN

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 8803652
SA 25325

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/03/89. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4663167	05-05-87	None		
FR-A- 2390159	08-12-78	NL-A- 7805005 BE-A- 866697 DE-A, C 2818655 JP-A- 53142514 AU-A- 3423578 GB-A- 1575343 US-A- 4311712 AU-B- 514644 CA-A- 1114758 SE-A- 8201350 SE-A- 8201351 US-A- 4370349 SE-A- 7805276 SE-B- 440725 CH-B- 650944 CH-B- 652615 SE-B- 453962 SE-B- 454049		14-11-78 03-11-78 23-11-78 12-12-78 20-09-79 17-09-80 19-01-82 19-02-81 22-12-81 04-03-82 04-03-82 25-01-83 11-11-78 19-08-85 30-08-85 29-11-85 21-03-88 28-03-88
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